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Interstitial Duplication 8q22-q24: Report of a Case Proven by FISH With Mapped Cosmid Probes

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We report on a 6-month-old malformed female infant with a de novo interstitial duplication of an 8q22-q24 segment. She had an excess dark-band on the 8q distal region by GTG-banded chromosome analysis, which was likely to be 8q23. We performed FISH analysis using cosmid probes mapped to 8q23 and proved that the patient had an 8q duplication including the 8q23 region.

KEY WORDS: chromosome aberration, 8q23, duplication 8q2, dup(8)(q22-q24), FISH, cosmid probe

INTRODUCTION

It is difficult to identify a small excess chromosome band, except for an individual with an unbalanced reciprocal translocation derived from a parent with balanced translocation. Even by the microdissection-chromosome painting method [Deng et al., 1992; Ohta et al., 1993], it is hard to detect a small interstitial excess band. FISH analysis using mapped cosmid probes is very useful for the identification of constitutional chromosome aberrations. We identified an 8q22-q24 duplication in a malformed patient by FISH using cosmid probes mapped to human chromosome area 8q23. To our knowledge, the duplication of this region has not been described previously.

MATERIALS AND METHODS

Clinical Report

The proposita was a 6-month-old girl. She was born at 40 weeks of gestation to a 29-year-old father and a 22-year-old mother, nonconsanguineous. Until the mother recognized her pregnancy at 5 months, she was taking some medicines for schizophrenia: alprazolam, sulpiride, amitriptyline, and etizolam. The infant's birthweight was 2,922 g. She was referred at age 1 month, because of convulsions. Clinical manifestations of the patient were hypertelorism, thin upper lip, small ears, short neck, wide-set nipples, hemangiomas over the left eyelid and left ear, and mild delay in developmental milestones. She was diagnosed as having epilepsy with an abnormal EEG. Laboratory studies demonstrated increased alkaline phosphatase levels in the blood (highest data: 2,230 IU/l), hypercalcemia (11.2 mg/dl) and hypophosphemia (6.6 mg/dl). Her TSH was 1.93 μIU/ml (0.46–3.7), free T3 was 5.1 pg/ml (3.34–5.26), free T4 was 1.6 ng/ml (0.97–1.66).

Cytogenetic Study

Chromosome preparations were obtained from cultured peripheral lymphocytes. High-resolution GTG-banding chromosome analysis was performed by the ethidium-bromide method [Ikeuchi and Sasaki, 1979].

Molecular-Cytogenetic Studies

FISH analysis was performed according to Takahashi et al. [1990], using three cosmid probes mapped to human chromosome area 8q23 (cCI8-1122, cCI8-1173, and cCI8-1185) [Nakamura et al., 1994]. Briefly, the patient's chromosomes were denatured in formamide and dehydrated with ethanol. Each cosmid DNA was labeled by enzymatic incorporation of biotin-dUTP by nick translation. After purification by ethanol precipitation, the biotinylated DNA was suspended in a formamide solution to which ~10-fold excess of human placental DNA was added. The denatured probes were then pipetted onto the chromosomes. The slides were incubated, washed, incubated again with avidin-FITC, and counterstained with propidium iodide in antibleach mounting medium. We observed signals on the chromosomes under an epifluorescence microscope.

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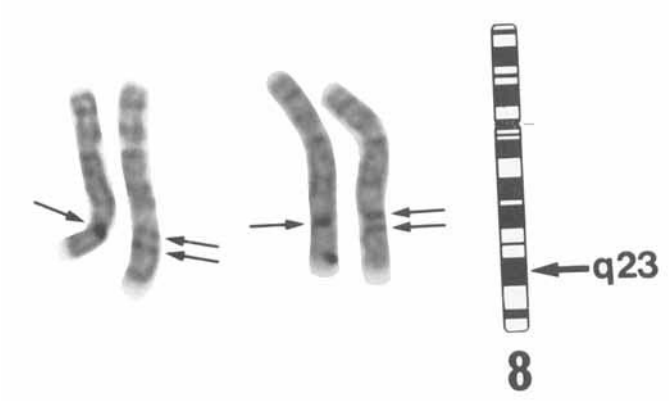

Fig. 1. G-banded partial ideograms of chromosome 8 of the patient. Arrows indicate an 8q23 band.

RESULTS

Cytogenetic Analysis

G-banding analysis revealed that the patient had an excess dark-and-light band on the 8q distal region (Fig. 1). The parents both had normal karyotypes. The excess dark-band was likely to be 8q23 band on the basis of the banding pattern.

FISH Studies

With each of two cosmid probes, cCI8-1122 and cCI8-1185, we detected one pair of signals of the probe on both homologous chromosomes 8 (Fig. 2A). But a study of cCI8-1173 showed one pair of signals on one chromosome 8 and two pairs on the other (Fig. 2B). Thus the patient was proved to have an 8q duplication including the 8q23 region.

DISCUSSION

In many kinds of chromosome aberrations, it is difficult to identify excess chromosome bands except for cases of derivative chromosome from a parental balanced translocation. We can suspect the origin of the excess bands only by banding patterns. In addition, several chromosome bands are necessary to show characteristic banding patterns. Many chromosome duplication cases seem to be unreported, because the excess bands cannot be identified. Recently, the molecular-cytogenetics technique has been developed, and reports of de novo chromosome duplication cases have increased using commercial based whole-chromosome-painting probes [Zelante et al., 1994; Chen et al., 1995; Conte et al., 1995; Zollino et al., 1995; Wiley et al., 1995; North et al., 1995; Rao et al., 1995]. However, most de novo duplications have an excess of bands originating from

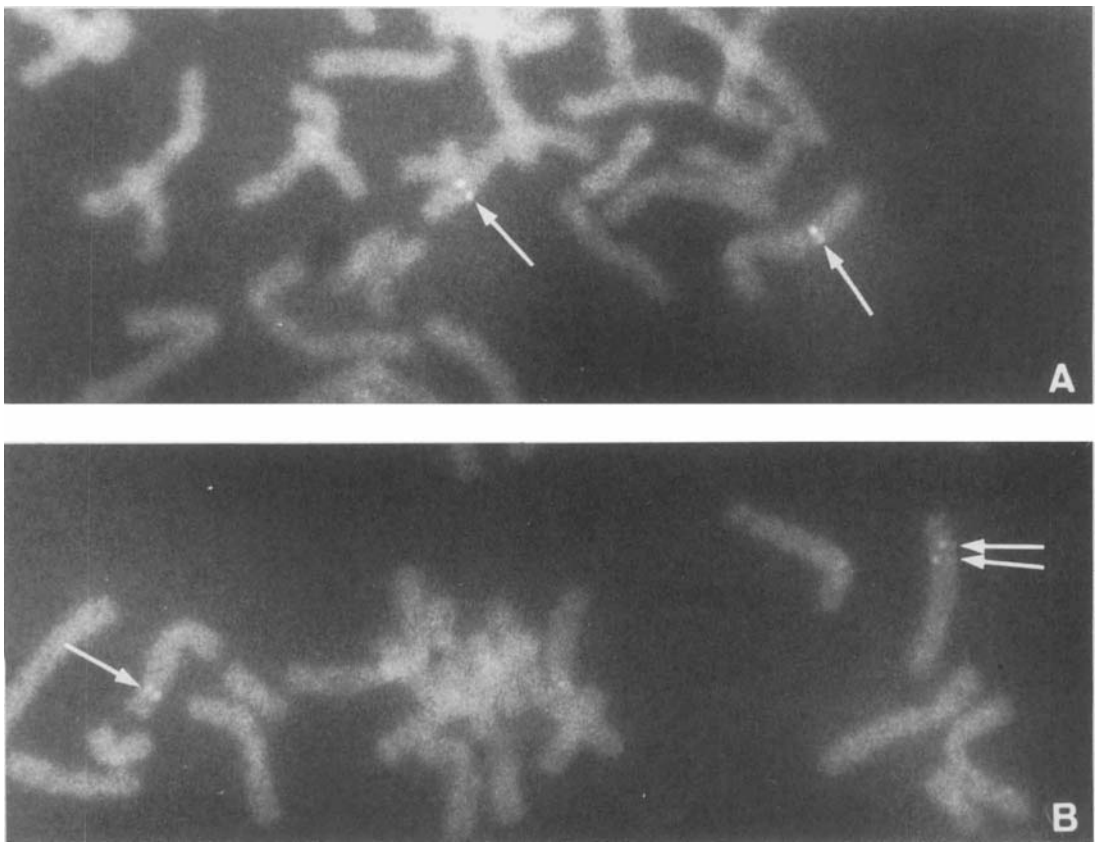

Fig. 2. FISH study of the patient using cosmid probes mapped to 8q23. Arrows indicate signals of cosmid probes. **A:** FISH with a cosmid probe (cCI8-1185). **B:** FISH with a cosmid probe (cCI8-1173).

TABLE I. Clinical Manifestations of Patients With Duplication of 8q22-q24

Findings	Walker and Bocian, 1987	Donnenfeld et al., 1990	Naritomi and Hirayama, 1989	Present case 1994
Duplicated segments	q22~q24-qter	q13-q24.1	q23.3-q24.13	q22-q24
Abnormal skull shape	7/9	+		
Ears	large:14/11	large		small
Abnormal pinnae	14/17	+	+	
Hypertelorism	18/21	+	+	±
Epicanthal folds			+	
Strabismus			+	
Broad nasal bridge			+	
Flat nasal root				+
Broad nasal root	10/20	+		
Anteverted nares	10/10	+		
Bulbous tip of the nose with thick alae nasi			+	
Long philtrum	7/14	+	+	
Thin upper lip	13/13	+		+
Highly arched/cleft palate	13/18	+		
Microretrognathia	8/10	+	+	
Clinodactyly	14/19	+		
Distal axial triradius	12/13	+		
Broad, short neck	8/9	+	+	+
Redundant skin			+	
Muscular hypotonia			+	
Widely spaced nipples	2/2			+
Kyphoscoliosis	7/10			
Cardiac malformation	18/21	+		
Cryptorchidism	9/9	+		
Short stature	18/23			
Hemangioma				+
Mental retardation	26/26		+	+
EEG abnormality	5/6			+

the same chromosome. In such cases, the chromosome with duplication was entirely painted by a whole chromosome painting probe, by which we could not identify the origin of the excess bands. Even by the microdissection-chromosome painting method [Deng et al., 1992; Ohta et al., 1993], it is hard to detect a small interstitial excess band such as this case. FISH analysis using a mapped cosmid probe is very useful for identification of constitutional chromosome aberrations [Aqua et al., 1995; Knoll et al., 1995; Lindsay et al., 1995; Stratton et al., 1995].

G-banding chromosome analysis of this patient revealed that she had a de novo excess dark-and-light band on the 8q distal region with a banding pattern that resembled 8q23. We performed FISH analysis using three cosmid probes mapped to 8q23. Fortunately, we detected two pairs of cosmid signals on the abnormal chromosome 8 using one of the probes (cCI8-1173). Thus the patient was proved to have a duplication including the 8q23 region. However, the other two probes (cCI8-1122, and cCI8-1185) were not included in the duplication.

Clinical manifestations of the previously reported cases with partial duplication of 8q22-q24 are shown in Table I. Among these cases, the only common manifestations of duplication 8q22-q24 are hypertelorism, thin upper lip, short neck, and mental retardation [Walker and Bocian, 1987; Naritomi and Hirayama, 1989; Donnenfeld et al., 1990].

The thyrotropin-releasing hormone (TRH) receptor gene has been assigned to 8q23 [Morrison et al., 1994]. Levels of TSH, free T3, and free T4 of our patient were normal. It remains to be seen whether the duplicated region of our patient did not include the TRH receptor gene locus, or whether the excess gene expression of the TRH receptor did not influence the TRH level.

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